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14. ABSTRACT
Abnormal levels of total creatine (tCr) in veterans with Gulf War Illness have been observed in prior studies. The goal of this research is to estimate amounts and ^{1}H transverse relaxation times (T2s) of the methyl peaks of the molecules phosphocreatine (PCr) and free creatine (Cr) in brains of ill and well control) Gulf War veterans using phosphorus (^{31}P) and proton (^{1}H) magnetic resonance spectroscopy (MRS). This will add more detailed and specific information about the previous preliminary reports of abnormal levels of these metabolites in brains of ill Gulf War veterans, validating this potential diagnostic marker and providing better understanding of underlying pathophysiology. Secondary goals are to measure amounts of adenosine triphosphate (ATP), inorganic phosphate (P_i), and magnesium ion (Mg^{2+}) and to estimate intracellular pH from ^{31}P MRS data. Year 1 progress and achievements included testing of a new dual-tuned ^{31}P-^{1}H head coil for 3T MRS, developing and optimizing protocols and parameters for ^{31}P and ^{1}H MRS, performing theoretical calculations using parameter estimates from the literature to characterize the creatine phosphokinase (CPK)-catalyzed chemical exchange of phosphate between PCr and Cr, and doing normal volunteer experiments to explore and determine the best 3T parameters for measuring bi-exponential ^{1}H T_2 relaxation of the methyl peak of tCr at 3.0 ppm.

15. SUBJECT TERMS
Gulf War Illness, brain creatine and phosphocreatine metabolism, ^{1}H and ^{31}P magnetic resonance spectroscopy (MRS), bi-exponential T_2 relaxation

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Introduction

The purpose of this research is to investigate creatine metabolism in brains of Gulf War Illness veterans classified into four groups of healthy controls and Haley Syndromes 1, 2, and 3 by estimating amounts of phosphocreatine (PCr) and free creatine (Cr) using ^{31}P (for basal ganglia and white matter centrum semiovale) and ^{1}H (for basal ganglia) magnetic resonance spectroscopy (MRS). Secondary goals are to measure ^{1}H T_2 relaxation times of the methyl resonances of PCr and Cr and to measure amounts of adenosine triphosphate (ATP), inorganic phosphate (P_i), and magnesium ion (Mg^{2+}) and to estimate intracellular pH from ^{31}P MRS data.

Body

Task 1 was completely successfully ahead of schedule in Q1, and Task 2 was completed successfully on schedule in Q2. Tasks for the project are about 7 months behind schedule because of the difficulties encountered in getting the ^{31}P MRS experiments to work well on the 3T Philips scanner with multinuclear capability at the Advanced Imaging Research Center (AIRC), the limited time available on that MR system, and (recently) delays caused by the P.I.'s impending move to Georgia State University in Atlanta on 11/01/13. A total of 33 normal volunteer candidates are now on the recruitment roster for the preparatory pilot study (Task 3d). An extensive series of tests was done to get the ^{31}P coil of the dual-tuned ^{31}P-^{1}H head coil to perform reliably; ^{31}P protocol sequences and parameters were developed, tested, and finalized. Sequences and parameters for the ^{1}H protocol were also developed, tested, and finalized. This included experiments to define the 3T parameters for which the difficult task of measuring bi-exponential T_2 relaxation of the methyl peak of tCr at 3.0 ppm are optimized.

Task 1. Revision of IRB and consent form to include USAMRMC ORP-specific language, including local IRB and DoD review and approval (**months 1-5**)

- This task was completed successfully, ahead of schedule, in Q1. In Q2, a modification to include the time as well as date for signature collection for the consent form and for the HIPAA authorization form was approved by the local IRB on 02/03/2013, and the first CR report was approved by the local IRB on 02/15/2013 and by the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO) on 03/01/2013; this approval will expire on 02/03/2014. By the end of Q4, responses to recruitment flyers had been received from 34 volunteer candidates for the 4 normal participants proposed for the preparatory pilot study (Task 3d).

Task 2. Ordering, design, construction, and delivery of Advanced Imaging Research (AIR) dual-tuned ^{31}P-^{1}H head coil (**months 1-5**)

- The dual-tuned ^{31}P-^{1}H head coil was ordered from AIR on 12/18/2012 and delivered on 02/06/2013; the annular loading phantom and holder for testing ^{31}P coil performance was delivered on 03/05/2013. Although the ^{1}H coil performance met specifications, no signal was detected with the ^{31}P coil upon initial testing, so the coil was returned to the vendor for repair. The quadrature combiner connections for ^{31}P were reversed and the modified coil was delivered on 03/15/2013. The modified ^{31}P coil was tested and again no signal was detected in either polarity/orientation in the magnet. Philips engineers, consulting with Cleveland headquarters where the coil was originally tested, tested the UT Southwestern AIRC 3T Philips MR system multi-

nuclear transmit and receive channels several times over the next 10 days, finally locating and correcting a cable connection problem. After again swapping the ^{31}P quadrature combiner connection to return them to their original configuration, the coil was again tested in the 3T magnet on 03/28/13 and this time the expected 31P signal was detected. However, intermittent problems continued over the following 3.5 months, so the coil was again returned to the vendor for repair on 07/19/13. An intermittent connection on a semi-rigid cable in the transmit-receive (TR) switching circuit of the ^{31}P receive chain was found and fixed. After the coil was shipped back on 08/06/13, phantom and human volunteer tests over the next three weeks indicated the intermittent problems were gone. These technical problems have delayed the project by about six months.

Task 3. Development/testing of 3T Philips protocols, sequences, and parameters (**months 1-6**)

3a. Plan and set up MR scan protocols and sequences and optimize parameters (**months 1-5**).

- Both ^{1}H and ^{31}P protocols, sequences, and parameters were set up, tested, modified and optimized, and finalized, including extensive testing of the dual-tuned coil, with phantoms and with normal human volunteers (see 3b-3d below).

3b. Make phantoms for both ^{1}H and ^{31}P MRS sequence testing and testing of the AIR dual-tuned ^{31}P-^{1}H head coil (**months 1-5**).

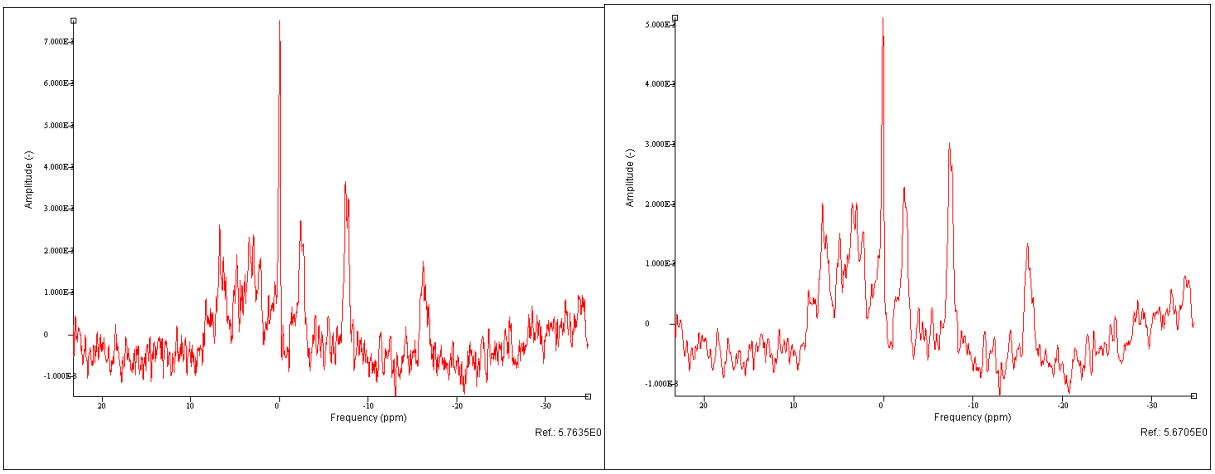
- To augment available Philips and AIR phantoms, a 250-mL phantom initially containing 30 mM PCr, 15 mM ATP, and 16.5 mM $MgCl_2$ in 2% BSA to realistically mimic brain metabolite spectra was made for calibrating and testing the ^{31}P-^{1}H head coil and Philips 14-cm diameter ^{31}P surface coil with ISIS spatial localization.
- To test ability to measure bi-exponential relaxation of the tCr peak with ^{1}H MRS in order to resolve Cr and PCr, four phantoms were made. Each consisted of 20 mL of 2% bovine serum albumin (BSA) aqueous solution buffered with 50 mM HEPES and (1) 50 mM Cr, (2) 50 mM PCr, (3) 50 mM Cr and 50 mM PCr, (4) 50 mM Cr, 60 mM PCr, 30 mM ATP, 33 mM $MgCl_2$, 3500 units or 0.42 mg/mL of creatine phosphokinase (CPK), ph = 7.4. The 20 mL vials were placed in a 125 mL bottle of 150 mM saline to reduce susceptibility artifacts near the vial walls and permit better shimming to narrower line widths and thus suppression of the water signal

3c. Collect phantom data to characterize and verify performance of the dual-tuned ^{31}P-^{1}H head coil, run final tests of protocols and sequences, do final parameter optimizations (**month 6**).

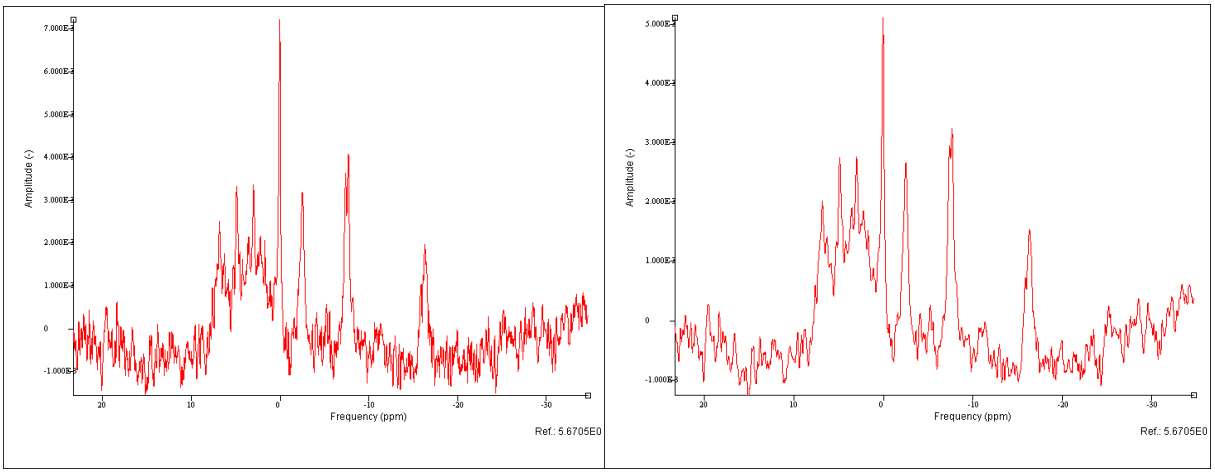
- Calibration and sensitivity tests were performed on the ^{1}H and ^{31}P circuits of the dual-tuned AIR ^{31}P-^{1}H head coil and compared with the Philips P140 14-cm ^{31}P surface coil, using both Philips-AIR and homebuilt phantoms.
- Although the ^{1}H sensitivity was only about half that specified by the manufacturer of the coil, the ^{1}H channel sensitivity was adequate for imaging to position voxels, for shimming to provide narrow line widths, and for decoupling to collapse ^{31}P-^{1}H spin-spin coupling in ^{31}P spectra. The 8-channel and 32-channel Philips ^{1}H head coils had better ^{1}H sensitivity, and were used for ^{1}H MRS data collection.
- The ^{31}P performance of the dual-tuned AIR-SREE coil typically met the limited manufacturer specifications, but intermittent sensitivity issues led to several rounds of trouble-shooting and repairs of both the coil and the MR scanner in a six-month period. Although performance is now reliable, our data indicate that the parameters in the coil configuration file may not be optimally calibrated.
- Nonetheless, adjustments and modifications have been made, and both ^{1}H and ^{31}P protocols and parameters are now finalized.

3d. Test the entire MRS protocol on 4 normal volunteers as a preparatory pilot study (**month 6**).

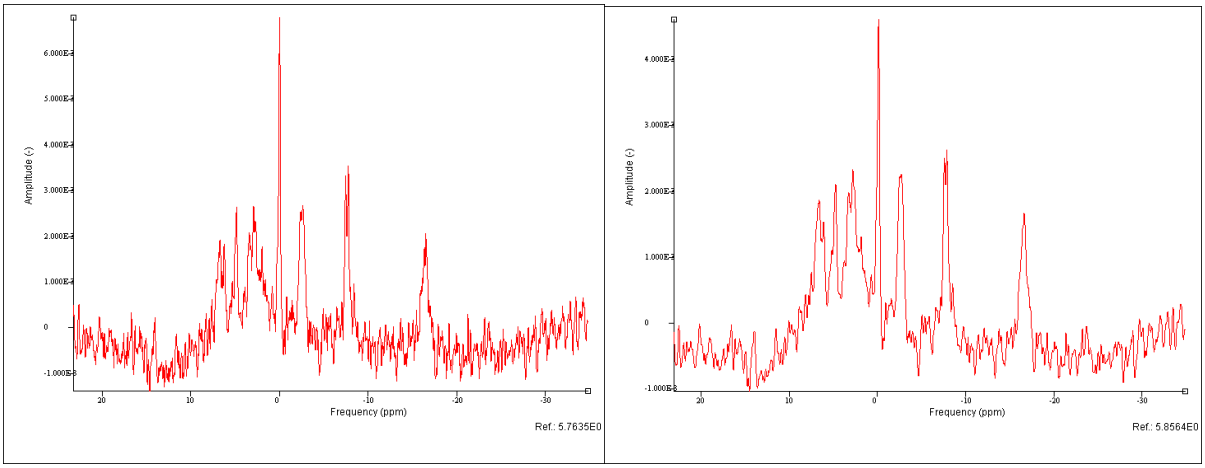
- In Q4, an additional 7 volunteer candidates responded to recruitment flyers, bringing the total on the recruitment roster available for the preparatory pilot study to 34.
- A total of 24 human protocols tests have been done, 12 with ^{1}H (one at 7T) and 12 with ^{31}P. Eleven human protocol tests were performed in Q3, four with ^{1}H and seven with ^{31}P, and thirteen were performed in Q4, seven with ^{1}H and five with ^{31}P at 3T and one with ^{1}H at 7T. Initial human protocol tests and those demanding a still, compliant subject for long multi-echo T_2 measurement tests were conducted with the P.I. as subject (twice in April, four times in May, three times in June, four times in July, four times in August). Seven other subjects were also used for protocol and parameter tests and optimizations.
- This phase of the project was completed successfully, albeit 5 months behind schedule, for both ^{31}P (see following bullet point and Sub-section A of "Problems" section below) and ^{1}H (see second bullet point below).
- *Final ^{31}P protocol and sequence parameters*: Even after fixing scanner and coil problems causing intermittent sensitivity deficiency, ^{31}P gave lower SNR performance than had been anticipated, which will necessitate using shorter TR (2-3 s) rather than the 20 s needed for complete T_1 relaxation and more signal averages. Furthermore, voxel volume will need to be at least 100 mL for adequate SNR in a reasonable time, which will preclude including basal ganglia as originally proposed. A bilateral white matter centrum semiovale voxel gives acceptable though not good 11-minute ^{31}P spectra under these conditions (see Figure 1 below).



4_1, TR = 2 s, NSA = 320, duration = 10:48, rms B_1 = 2.35 μT

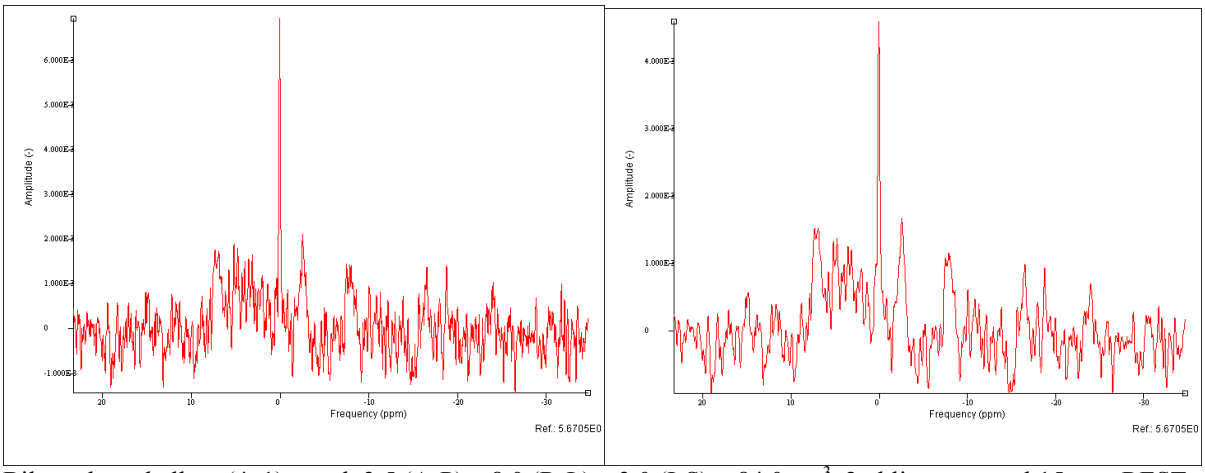


5_1, TR = 3s, NSA = 216, duration = 10:57, rms B_1 = 1.92 µT

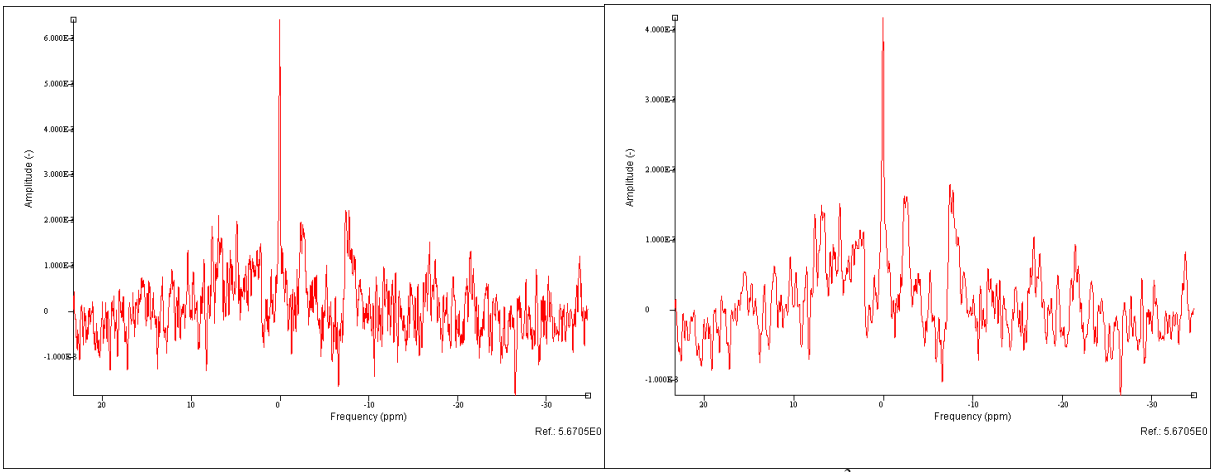


6_1, TR = 2 s, NSA = 320, duration = 10:48, rms B_1 = 2.35 µT

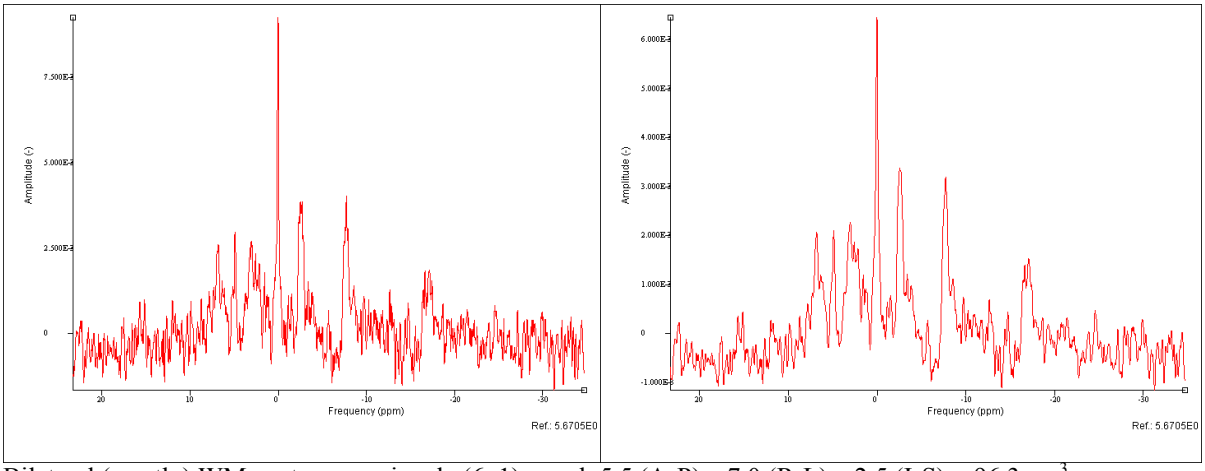
Figure 1. 08/09/13 3T6594 ^{31}P ISIS MRS spectra using AIR/SREE dual-tuned head coil (after repair). Bilateral (mostly) white matter (WM) centrum semiovale, 5.5 (A-P) x 7.0 (R-L) x 2.5 (I-S) = 96.3 cm^3, 4 disdacqs, TE = min (0.20 ms), offset frequency = -250 Hz, SW = 3000 Hz, 2048 data points zero-filled to 4096, RO duration = 682.7 ms, broadband WALTZ decoupling (max B1 = 3 µT, offset frequency = -100 Hz), LB = 15 Hz (left) and 30 Hz (right). Tallest peak is PCr, three right-most peaks are ATP.



Bilateral cerebellum (4_1) voxel, 3.5 (A-P) x 8.0 (R-L) x 3.0 (I-S) = 84.0 cm^3, 2 oblique coronal 15-mm REST slabs.



Bilateral frontal_ACC (5_1) voxel, 4.0 (A-P) x 6.0 (R-L) x 3.0 (I-S) = 72.0 cm^3, 2 oblique axial 15-mm REST slabs.



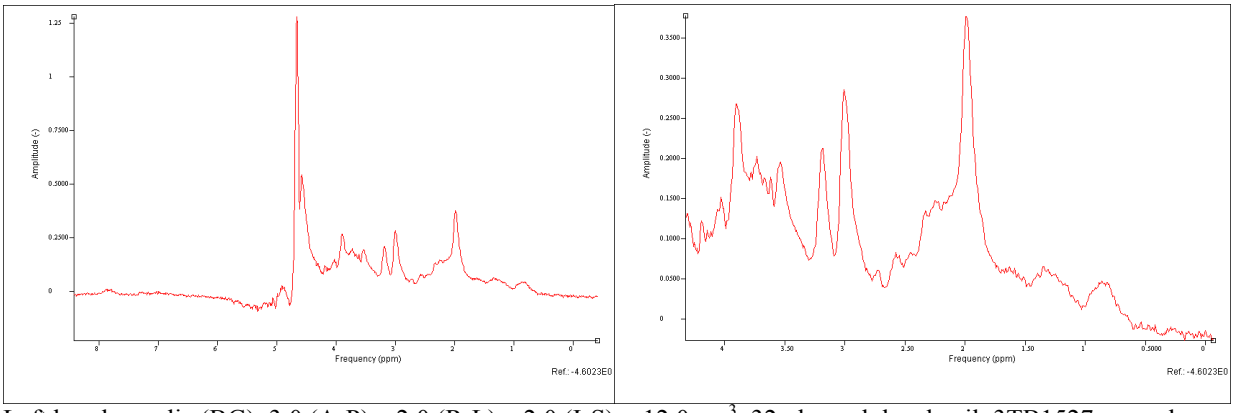
Bilateral (mostly) WM centrum semiovale (6_1) voxel, 5.5 (A-P) x 7.0 (R-L) x 2.5 (I-S) = 96.3 cm^3

Figure 2. 08/07/13 3T6586 ^{31}P ISIS MRS using AIR/SREE dual-tuned head coil (after repair). TR = 4 s and NSA = 144, duration = 9:44, TE = min (0.10 ms), 2 disdacqs, SW = 3000 Hz, 2048 data points zero-filled to 4096, LB = 15 Hz (left) and 30 Hz (right). Tallest peak is PCr, three right-most peaks are ATP.

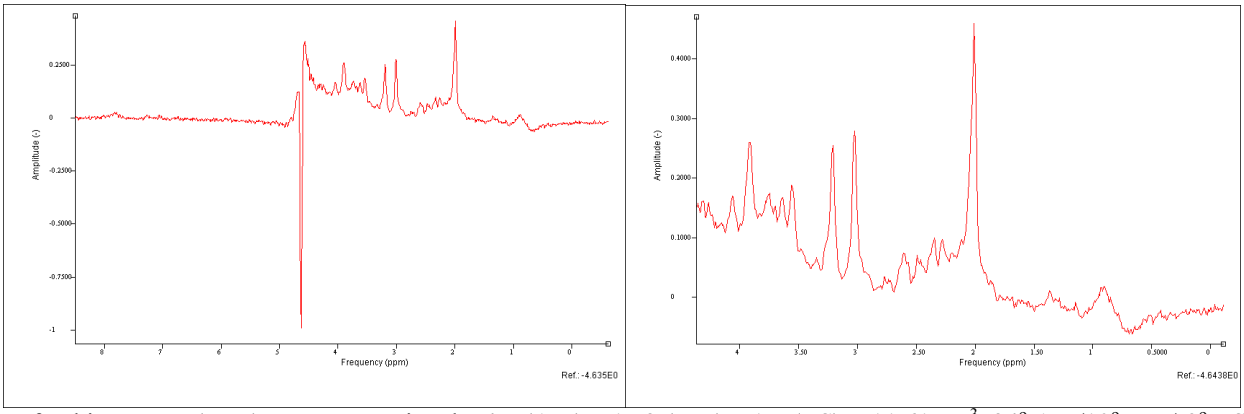
But 10-minute ^{31}P spectra obtained from slightly smaller voxels of bilateral frontal lobe including anterior cingulate cortex (ACC) and of bilateral cerebellum (see Figure 2) were of lower quality. The rationale for frontal lobe was that Lac and Glx change there with exercise differs in two groups of ill Gulf War veterans (GWV) with chronic fatigue syndrome (CFS) that respond differently to exercise stress (1). The rationale for cerebellum was that only striatum and hippocampus have higher acetylcholinesterase (AChE) activity in rat brain, from an organophosphate (OP) toxicity study (2).

- *Final ^{1}H protocol and sequence parameters*: Sequences for ^{1}H MRS data were tested on human volunteers with the 8-channel and 32-channel Philips head coils on the two 3T Philips MR scanners. The two coil types gave similar signal-to-noise ratio (SNR). Using both scanners increases the scheduling flexibility for ^{1}H scans. Using a water saturation bandwidth of 75 Hz, it was determined that MOIST water suppression worked slightly better and more reliably than either VAPOR or CHESS methods. Voxel sizes and locations and number of spectral averages (NSA) needed to obtain spectra with adequate SNR within the 1.25 hours allotted for the ^{1}H session were tested and verified. The TR of 8 s provides fully relaxed spectra with no dependence of signal intensity upon T_1 for both metabolites and water. The short echo time (TE = 30 ms) means there is also negligible effect of T_2 on metabolite quantification. The voxels to be interrogated are: **(1)** left basal ganglia, 12 mL, (30 mm A-P x 20 mm R-L x 20 mm I-S), NSA = 64, duration = 8:32; **(2)** left white matter centrum semiovale, 12 mL (45 mm A-P x 18 mm R-L x 15 mm I-S), NSA = 32, duration = 4:16; **(3)** left anterior cingulate, 6 mL (40 mm A-P x 13 mm R-L x 12 mm I-S), NSA = 64, duration = 8:32; **(4)** left cerebellum, nominally 22.5 mL (25 mm A-P x 30 mm R-L x 30 mm I-S), NSA = 24, duration = 3:12. These times do not include placement of voxel positions, shim and water suppression optimization, and collection of non-suppressed water reference spectra, which bring the experiment duration to slightly more than an hour. Two cerebellum voxels were initially tested, one mainly white matter and one mainly gray matter, but difficulties in reliably shimming to a narrow Lorentzian line and effectively suppressing water signal in the multiply oblique

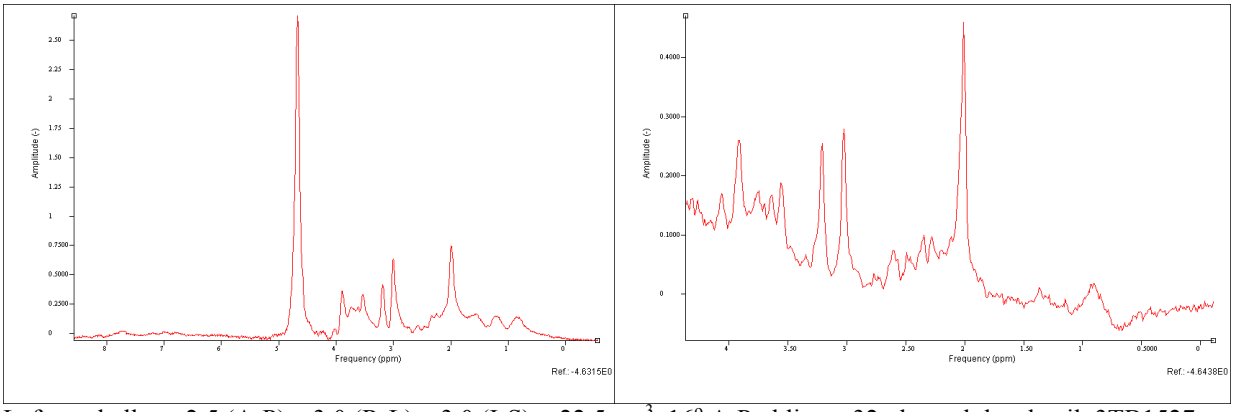
voxels, as well as time constraints, led to the conclusion that a single cerebellum voxel would be more practical. Representative ^{1}H spectra from these four brain regions are shown in Figure 3 below. Good water suppression (see suppressed water peak intensities at 4.6 ppm in left panels of Figure 3) and metabolite SNR were routinely obtained.



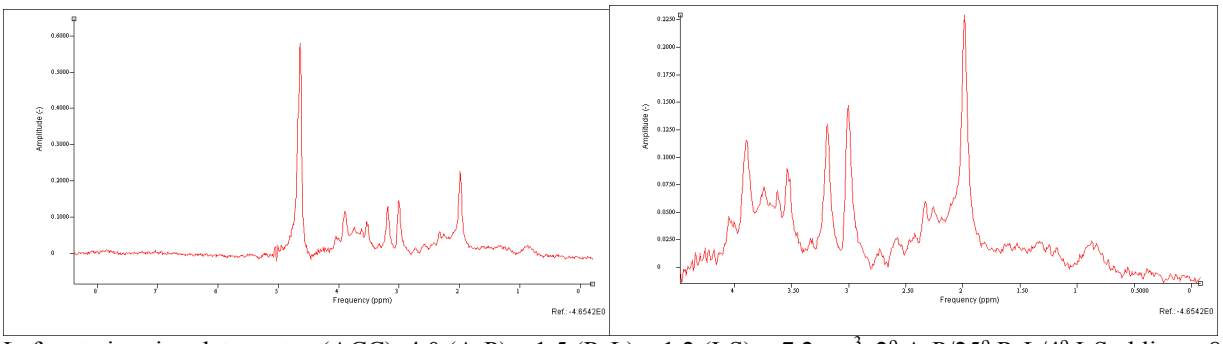
Left basal ganglia (BG), 3.0 (A-P) x 2.0 (R-L) x 2.0 (I-S) = 12.0 cm^3, 32-channel head coil, 3TB1527 normal control (NC) volunteer (07/12/13), NSA = 64, t_{acq} = 8:32



Left white matter (WM) centrum semiovale, 4.5 (A-P) x 1.72 (R-L) x 1.5 (I-S) = 11.61 cm^3, 34° A-P/10° R-L/-9° I-S oblique, 32-channel head coil, 3TB1527 normal control (NC) volunteer (07/12/13), NSA = 32, t_{acq} = 4:16



Left cerebellum, 2.5 (A-P) x 3.0 (R-L) x 3.0 (I-S) = 22.5 cm^3, 16° A-P oblique, 32-channel head coil, 3TB1527 normal control (NC) volunteer (07/12/13), NSA = 24, t_{acq} = 3:12



Left anterior cingulate cortex (ACC), 4.0 (A-P) x 1.5 (R-L) x 1.2 (I-S) = 7.2 cm^3, 2° A-P/25° R-L/4° I-S oblique, 8-channel head coil, 3T6463 normal control (NC) volunteer (06/20/13), NSA = 64, t_{acq} = 8:32

Figure 3. Typical 3T 1H MRS from four brain regions obtained with TR = 8 s, TE = 31-32 ms, SW = 2 kHz, 1024 →2048points, 75 Hz MOIST water suppression, LB = 3Hz. Left panels show water suppression quality, right panels are expanded to show only metabolite signals. Methyl peaks of NAA (2.0 ppm), Cr (3.0 ppm), and Cho (3.2 ppm) are most prominent.

The rationale for including WM centrum semiovale is that white matter damage has been reported in Gulf War Illness (GWI) (3-6). The rationale for including ACC is that Lac and Glx changes with exercise differ in two groups of ill GWV with CFS that respond differently to exercise stress (1). The rationale for adding cerebellum is that only striatum and hippocampus have higher AChE activity in rat brain, from an organophosphate (OP) toxicity study (2).

- *Experimental results for bi-exponential T_2 for tCr (and other) peaks*: Multi-TE experiments were done with normal volunteers to determine if bi-exponential decay curves for the total creatine (tCr, or PCr + Cr) methyl peak could be observed, as reported in one paper in the literature (7). Most data were collected from a white matter voxel in left hemisphere centrum semiovale, to ensure that bi-exponential decay from tCr in gray matter and tCr in white matter was not mistaken for bi-exponential decay from PCr and Cr. Some data were collected from a parietal voxel containing nearly equal amounts of gray matter and white matter, to see if bi-exponential behavior of tCr due to tissue type could be detected (8). At 1.5T, T_2 has been reported to be longer in WM than in GM for NAA (483 ± 20 ms cp. 399 ± 9 ms), similar in WM and GM for tCr (209 ± 5 ms cp. 204 ± 2 ms), and shorter in WM than in GM for tCho (325 ± 10 ms cp. 401 ± 20 ms) (8). To check the effect of differing minimum TE values on the ability to detect and quantify the faster relaxing component, minimum TE was varied between 30 and 50 ms using a PRESS localization sequence and a few experiments were done with STEAM localization to lower the minimum TE still further, to 10 ms. The effect of minimum TE on fit results was also investigated by systematically ignoring initial (short) TE values in the fitting procedure. Longest TE values were selected to ensure that signal had decayed nearly completely, to improve the fit quality for the longer T_2 component. To check if inadequate signal-to-noise might lead to fitting of a very long T_2 component artifact from the long-TE tail of the decay curve, a noise floor or baseline asymptote parameter was included in some of the fits. Finally, since T_2 values were expected to become shorter at higher field strength, and our 3T data were being evaluated in light of prior literature work reporting distinguishably different PCr and Cr T_2 values at 1.5 T (7) but not at 4T (9), a multiple-TE experiment was also conducted at 7T.

Relaxation data in Tables 1-5 were collected at 3T with 32 signal averages and TR = 3.0 s and required about 75 minutes. Using jMRUI, time-domain FIDs were zero-filled and multiplied with an exponential function yielding 3 Hz Lorentzian broadening before Fourier transformation and phasing. Table 1 shows T_2 values calculated for the 3.0 ppm tCr` methyl peak region with a 1-exponent, 2-parameter fitting equation ($y = ae^{-bx}$) and a 2-exponent, 4-parameter fitting equation ($y = ae^{-bx} + ce^{-dx}$) and, in the rows shaded in gray, with 1-exponent, 3-parameter fitting equation ($y = y_0 + ae^{-bx}$) and a 2-exponent, 5-parameter fitting equation ($y = y_0 + ae^{-bx} + ce^{-dx}$), where y_0 is a baseline asymptote or noise floor parameter.

Table 1. 3T T_2 values of total creatine (tCr) methyl peak at 3.0 ppm from single-exponential fit [e] and a double-exponential fit [f] of peak intensity decay curves from normal human volunteer subjects.

Subject	Date	Voxel [d]	TE Values	1-exponent	2-exponent		
				tCr T_2 /ms [a/y_0]	tCr T_{2A} /ms (f_A)	tCr T_{2B} /ms (f_B)	$(a+c)/y_0$
1 (M, 62y)	07/10/13	WM centrum semiovale 4.0x2.0x2.0 = 16.0 cm^3	40 log-spaced, 50-700 ms	155.2 ± 2.5	147.0 ± 10.9 (98.3%)	$1x10^8$ ± $4.5x10^7$% (1.7%)	
1 (M, 62y)	07/10/13	WM centrum semiovale 4.0x2.0x2.0 = 16.0 cm^3	40 log-spaced, 50-700 ms	147.0 ± 4.7 [57]	147.0 ± 0.0002 (57.3%)	147.0 ± 0.0002 (42.7%)	57
1 (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	44 log-spaced, 35-900 ms	146.9 ± 2.1	281.8 ± 228.4 (20.6%)	116.9 ± 28.0 (79.4%)	
1 (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	44 log-spaced, 35-900 ms	135.2 ± 3.3 [e] [45]	142.5 ± 5.4 [f] (51.9%)	11.5 ± 8.3 [f] (48.1%)	102.5
2 [a] (F, 41y)	07/19/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 32-700 ms	174.2 ± 4.0	174.2 ± 0.02 (58.1%)	174.2 ± 0.02 (41.9%)	
2 [a] (F, 41y)	07/19/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 32-700 ms	177.0 ± 8.6 [170]	177.0 ± $6x10^5$% (53.9%)	177.0 ± $7x10^5$% (46.1%)	170
3 [b] (F, 51y)	07/19/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	40 log-spaced, 32-700 ms	157.1 ± 4.1	130.75 ± 6.5 (94.4%)	$9x10^6$ ± $3x10^8$% (5.6%)	
3 [b] (F, 51y)	07/19/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	40 log-spaced, 32-700 ms	130.7 ± 6.1 [e] [17]	130.7 ± 0.03 [f] (56.25%)	130.7 ± 0.001 [f] (43.75%)	17
1 [c] (M, 62y)	07/26/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 10-600 ms	175.0 ± 6.0	244.1 ± 27.8 (56.3%)	23.6 ± 8.0 (43.7%)	
1 [c] (M, 62y)	07/26/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 10-600 ms	113.5 ± 13.8 [e] [8]	284 ± 120 [f] (55.9%)	24.6 ± 9.5 [f] (44.1%)	30
1 [c] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 10-600 ms	146.2 ± 4.6	272.8 ± 126.8 (38.35%)	85.8 ± 27.9 (61.65%)	
1 [c] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 10-600 ms	122.0 ± 6.1 [e] [36]	329 ± 1008 [f] (33.8%)	89.5 ± 56.9 [f] (66.2%)	69.5

[a] Subject uncomfortable, talking, and moving intermittently last 15 minutes of scan; poor quality in 25% of data.
[b] Subject uncomfortable last 10 minutes of scan, stopped with 3 minutes left for a restroom break, did not get data for last two TE values.
[c] PRESS used for localization on all except 07/26/13 and 08/16/13 Subject #1 scans, when STEAM was used.
[d] All voxels in left hemisphere.
[e] Used a 1-exponent, 2-parameter fit ($y = ae^{-bx}$) in odd rows and a 1-exponent, 3-parameter fit ($y = y_0 + ae^{-bx}$) in even, gray-shaded rows; signal-to-noise = a/y_0 in the latter.
[f] Used a 2-exponent, 4-parameter fit ($y = ae^{-bx} + ce^{-dx}$) in odd rows and a 2-exponent, 5-parameter fit ($y = y_0 + ae^{-bx} + ce^{-dx}$) in even, gray-shaded rows; signal-to-noise = $(a + c)/y_0$ in the latter.

In one case (Subject 2), the bi-exponential fit yielded components with the same T_2 value. In two cases (Subject 3 and 07/10/13 session of Subject 1), the second component either constituted only a small percentage of the total 3.0 ppm signal intensity and had an

unrealistically long T_2 value (without the extra y_0 parameter) or had the same T_2 value as the first component (with the extra y_0 parameter). In the remaining three cases, the second component was a substantial fraction (44-79%) of the total 3.0 ppm signal intensity, with shorter T_2 values of 12-117 ms, not dissimilar from Ke et al. 2002 (7). Two of these three cases were those in which the shortest TE was 10 ms, achieved with STEAM localization, 20 ms shorter than achievable with PRESS localization. Including a baseline asymptote or noise floor parameter in the one- and two-exponential fit equations significantly shortened the calculated T_2 of both components for the single PRESS experiment where bi-exponential behavior was observed, but had little effect (both component T_2 values slightly lengthened) for the two STEAM experiments with shorter minimum TE.

The effect of minimum TE in PRESS data was also investigated by systematically ignoring initial (short) TE values in the fitting procedure (Table 2). This produced longer single-exponential T_2 values. For bi-exponential fits, ignoring the shortest TE datum of 35 ms lengthened the calculated T_2 for both components, but omitting data for TE values of 40 ms and longer resulted in replacement of the shorter-T_2 second component with an unrealistically long T_2 component comprising only a few percent of the total 3.0 ppm signal intensity and a concomitant shortening of the first component T_2 to values near those obtained with a single-exponential equation fit. This is similar to the result obtained in the 07/10/13 session of Subject 1 (Table 1), with minimum TE of 50 ms. It indicates that TE values shorter than 40 ms are needed to observe bi-exponentiality here.

Table 2. 3T T_2 values of total creatine (tCr) methyl peak at 3.0 ppm from single- and double-exponential fits of peak intensity decay curves, ignoring initial data points, from PRESS of normal volunteer subject.

Subject	Date	Voxel [e]	TE Values	1-exp, 2-par	2-exp, 4-par	
				tCr T_2 /ms	tCr T_{2A} /ms (f_A)	tCr T_{2B} /ms (f_B)
1 (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	44 log-spaced, 35-900 ms	146.9 ± 2.1	281.8 ± 228.4 (20.6%)	116.9 ± 28.0 (79.4%)
1 [a] (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	43 log-spaced, 40-900 ms	147.7 ± 2.2	373.4 ± 478.0 (11.8%)	124.7 ± 22.7 (88.2%)
1 [b] (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	42 log-spaced, 44-900 ms	149.9 ± 2.1	139.4 ± 1.5 (97.9%)	$9.5x10^9 \pm 5x10^8$% (2.1%)
1 [c] (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	41 log-spaced, 49-900 ms	151.5 ± 2.0	142.0 ± 4.0 (98.1%)	$9.9x10^8 \pm 9x10^7$% (1.9%)
1 [d] (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	40 log-spaced, 54-900 ms	152.6 ± 2.1	143.5 ± 3.8 (98.3%)	$8.8x10^9 \pm 2.5x10^8$% (1.7%)

[a] Ignoring shortest TE value (TE = 35 ms) data.
[b] Ignoring two shortest TE values (TE = 35 ms, 40 ms).
[c] Ignoring three shortest TE values (TE = 35 ms, 40 ms, 44 ms).
[d] Ignoring four shortest TE values (TE = 35 ms, 40 ms, 44 ms, 49 ms).
[e] Voxel in left hemisphere.

The effect of minimum TE in STEAM data was also investigated by systematically ignoring initial (short) TE values in the fitting procedure (Table 3). This produced little change in single-exponential T_2 values until TE values of 30-50 ms were excluded, whereupon calculated T_2 values progressively lengthened. For bi-exponential fits, ignoring the shortest TE data shortened the long-T_2 component, increasing its contribution as TEs of 10-20 ms were successively ignored and then decreasing it as TEs of 20-40 ms were ignored. When the minimum TE was greater

than 50 ms, a single exponential result was approached, with a small residual component with an unrealistically long T_2.

Table 3. 3T T_2 values of total creatine (tCr) methyl peak at 3.0 ppm from single- and double-exponential fits of peak intensity decay curves, ignoring initial data points, from STEAM of normal volunteer subject.

Subject	Date	Voxel [i]	TE Values	1-exp, 2-par	2-exp, 4-par	
				tCr T_2 /ms	tCr T_{2A} /ms (f_A)	tCr T_{2B} /ms (f_B)
1 (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 10-600 ms	146.2 ± 4.6	272.8 ± 126.8 (38.35%)	85.8 ± 27.9 (61.65%)
1 [a] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	39 log-spaced, 15-600 ms	145.7 ± 4.8	227.8 ± 55.6 (51.6%)	65.6 ± 22.9 (48.4%)
1 [b] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	38 log-spaced, 20-600 ms	146.7 ± 5.2	209.8 ± 35.45 (57.4%)	52.7 ± 19.45 (42.6%)
1 [c] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	37 log-spaced, 25-600 ms	147.8 ± 5.5	193.2 ± 19.9 (59.6%)	36.2 ± 13.2 (40.4%)
1 [d] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	36 log-spaced, 30-600 ms	150.0 ± 5.9	184.3 ± 12.9 (51.6%)	23.9 ± 8.4 (48.4%)
1 [e] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	35 log-spaced, 36-600 ms	154.9 ± 6.1	182.3 ± 12.1 (44.6%)	20.2 ± 8.7 (55.4%)
1 [f] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	34 log-spaced, 42-600 ms	158.4 ± 6.5	180.5 ± 9.7 (18.7%)	13.3 ± 5.8 (81.3%)
1 [g] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	33 log-spaced, 47-600 ms	163.4 ± 6.8	183.3 ± 10.2 (15.2%)	13.3 ± 6.6 (84.8%)
1 [g] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	32 log-spaced, 53-600 ms	171.4 ± 6.3	144.05 ± 45.1 (93.2%)	1133 ± 8350 (6.8%)

[a] Ignoring shortest TE value (TE = 10 ms) data.
[b] Ignoring two shortest TE values (TE = 10 ms, 15 ms).
[c] Ignoring three shortest TE values (TE = 10 ms, 15 ms, 20 ms).
[d] Ignoring four shortest TE values (TE = 10 ms, 15 ms, 20 ms, 25 ms).
[e] Ignoring five shortest TE values (TE = 10 ms, 15 ms, 20 ms, 25 ms, 30 ms).
[f] Ignoring six shortest TE values (TE = 10 ms, 15 ms, 20 ms, 25 ms, 30 ms, 36 ms).
[g] Ignoring seven shortest TE values (TE = 10 ms, 15 ms, 20 ms, 25 ms, 30 ms, 36 ms, 42 ms).
[h] Ignoring eight shortest TE values (TE = 10 ms, 15 ms, 20 ms, 25 ms, 30 ms, 36 ms, 42 ms, 47 ms).
[i] Voxel in left hemisphere.

For comparison and to serve as controls, methyl peaks of tCho at 3.2 ppm (Table 4) and NAA at 2.0 ppm (Table 5) were analyzed in the same way as for tCr (Table 1).

For tCho (Table 4), in three cases (Subjects 2 and 3 and the 07/10/13 session of Subject 1), the bi-exponential fit yielded components with the same T_2 value. In the remaining three cases, the second component was a substantial fraction (31-92%) of the total 3.2 ppm signal intensity, with shorter T_2 values of 8-99 ms. Two of these three cases were those in which the shortest TE was 10 ms, achieved with STEAM localization, 20 ms shorter than achievable with PRESS localization.

For NAA (Table 5), in two cases (Subject 2 and the 07/10/13 session of Subject 1), the bi-exponential fit yielded components with the same T_2 value. Since the primary co-resonant 2.0 peak is a small contribution from NAAG, this was expected. In the remaining four cases, the second component was a substantial fraction (38-84%) of the total 3.2 ppm signal intensity, with shorter T_2 values of 5-17 ms. Two of these four cases were those in which the shortest TE was 10 ms, achieved with STEAM localization, 20 ms shorter than achievable with PRESS localization.

Table 4. 3T T_2 values of total choline (tCho) methyl peak at 3.2 ppm from single- and double-exponential fits of peak intensity decay curves from normal human volunteer subjects.

Subject	Date	Voxel [d]	TE Values	1-exp, 2-par	2-exp, 4-par	
				tCho T_2 /ms	tCho T_{2A} /ms (f_A)	tCho T_{2B} /ms (f_B)
1 (M, 62y)	07/10/13	WM centrum semiovale 4.0x2.0x2.0 = 16.0 cm^3	40 log-spaced, 50-700 ms	206.05 ± 3.55	206.06 ± 2.4x10^6% (54.5%)	206.06 ± 2.4x10^6% (45.5%)
1 (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	44 log-spaced, 35-900 ms	222.9 ± 3.3	234.2 ± 6.5 (7.7%)	7.6 ± 3.5 (92.3%)
2 [a] (F, 41y)	07/19/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 32-700 ms	221.1 ± 7.5	221.1 ± 6.5x10^5% (63.2%)	221.1 ± 6.5x10^5% (36.8%)
3 [b] (F, 51y)	07/19/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	40 log-spaced, 32-700 ms	247.8 ± 8.8	247.8 ± 9.5x10^5% (78.9%)	247.8 ± 9.5x10^5% (21.1%)
1 [c] (M, 62y)	07/26/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 10-600 ms	217.5 ± 15.0	266.6 ± 35.7 (68.8%)	24.4 ± 16.0 (31.2%)
1 [c] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 10-600 ms	168,5 ± 5.8	350.5 ± 211.5 (35.7%)	98.6 ± 33.4 (64.3%)

[a] Subject uncomfortable, talking, and moving intermittently last 15 minutes of scan; poor quality in 25% of data.
[b] Subject uncomfortable last 10 minutes of scan, stopped with 3 minutes left for a restroom break, did not get data for last two TE values.
[c] PRESS used for localization on all except 07/26/13 and 08/16/13 Subject #1 scans, when STEAM was used.
[d] All voxels in left hemisphere.

Table 5. 3T T_2 values of N-acetylaspartate (NAA) methyl peak at 2.0 ppm from single- and double-exponential fits of peak intensity decay curves from normal human volunteer subjects.

Subject	Date	Voxel [d]	TE Values	1-exp, 2-par	2-exp, 4-par	
				tCr T_2 /ms	NAA T_{2A} /ms (f_A)	NAA T_{2B} /ms (f_B)
1 (M, 62y)	07/10/13	WM centrum semiovale 4.0x2.0x2.0 = 16.0 cm^3	40 log-spaced, 50-700 ms	285.0 ± 0.9	285.0 ± 1x10^7% (51.4%)	285.0 ± 1x10^7% (48.6%)
1 (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	44 log-spaced, 35-900 ms	214.3 ± 6.2	238.8 ± 3.9 (15.95%)	10.9 ± 4.9 (84.05%)
2 [a] (F, 41y)	07/19/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 32-700 ms	268.0 ± 9.3	268.0 ± 1.6x10^7% (54.1%)	268.0 ± 1.6x10^7% (45.9%)
3 [b] (F, 51y)	07/19/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm^3	40 log-spaced, 32-700 ms	184.1 ± 8.6	227.95 ± 7.25 (21.7%)	13.8 ± 2.0 (78.3%)
1 [c] (M, 62y)	07/26/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 10-600 ms	364.3 ± 38.1	425.7 ± 40.4 (17.3%)	4.7 ± 1.8 (82.7%)
1 [c] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm^3	40 log-spaced, 10-600 ms	214.2 ± 8.7	262.8 ± 6.1 (61.9%)	17.1 ± 1.9 (38.1%)

[a] Subject uncomfortable, talking, and moving intermittently last 15 minutes of scan; poor quality in 25% of data.
[b] Subject uncomfortable last 10 minutes of scan, stopped with 3 minutes left for a restroom break, did not get data for last two TE values.
[c] PRESS used for localization on all except 07/26/13 and 08/16/13 Subject #1 scans, when STEAM was used.
[d] All voxels in left hemisphere.

Optimal data conditions for successful and reliable fitting of bi-exponential data have been explored and defined (10-12) and determined to be a signal contribution ratio of 1:1, at least a three-fold difference in relaxation times, and a signal-to-noise ratio (SNR) of at least 20 and preferably 50 or more. A logarithmic rather than linear spacing of TE values is preferred (12-14) as more efficient. The in vivo brain PCr:Cr ratio is near unity (7; 15-18). For the shortest TE of our 3T ^{1}H data for these six subjects, the SNR of the 3.0 ppm methyl resonances of tCr was 26 ± 8 (range 18-41), the SNR of the 3.2 ppm methyl resonances of tCho was 20.5 ± 6.4 (range 12-

28), and the SNR of the 2.0 ppm methyl resonance of NAA was 47.5 ± 10.0 (range 36-65). Thus bi-exponential fitting of PCr and Cr components with relaxation times differing threefold or more should be possible for tCr. Bi-exponential fitting should be possible for tCho and quite reliable for NAA. It has been speculated that a minimum of 40 TE values and preferably 60 or more are needed for reliable bi-exponential curve fitting (9). When separate T_2s were reported for PCr and Cr at 1.5T (7), 64 TE values (minimum 48 ms), 4 signal averages, and 27 cm^3 voxels were used. When only a single tCr T_2 was observed at 4T (9), 48 TE values (minimum 30 ms), 8 or 16 signal averages, and 8 cm^3 voxels were used. Although SNR values were not reported in these two papers, it is likely that SNR rather than number of TE values used was limiting in the later paper (9) with much smaller voxels. To partially test this, data from Figure 4 of Ke et al. 2002 (7) were re-analyzed, using all, half, and one-third of the data points. This figure shows the signal decay curve as a function of TE for a representative single subject, to demonstrate the bi-exponential decay of tCr and the mono-exponential decay of NAA. The re-analysis results are shown in Table 6 below.

Table 6. T_2 values of total creatine (tCr) and of Cr and PCr methyl peaks at 3.0 ppm from single- and double-exponential fits, respectively, of peak intensity decay curves from 1.5T data of Figure 4 of Ke et al., 2002 (7), with 4 signal averages and 27 cm^3 voxels.

TE Values	1-exp, 2-par	2-exp, 4-par	
	tCr T_2 /ms	tCr (Cr) T_{2A} /ms (f_A)	tCr (PCr) T_{2B} /ms (f_B)
64 (48-678 ms, 10 ms increments)	194.0 ± 4.7	243.3 ± 9.8 (47.2%)	44.1 ± 7.4 (52.8%)
32 (48-668 ms, 20 ms increments)	189.6 ± 7.1	253.0 ± 17.1 (45.7%)	49.4 ± 10.6 (54.3%)
32 (58-678 ms, 20 ms increments)	199.2 ± 6.2	234.3 ± 11.6 (46.7%)	37.1 ± 11.7 (53.3%)
22 (48-678 ms, 30 ms increments)	189.9 ± 8.6	235.8 ± 12.4 (43.2%)	33.8 ± 9.0 (56.8%)
21 (58-658 ms, 30 ms increments)	194.4 ± 7.4	244.0 ± 17.4 (49.8%)	49.3 ± 14.7 (50.2%)
21 (68-668 ms, 30 ms increments)	201.9 ± 8.5	390.2 ± 219.7 (27.9%)	111.8 ± 39.9 (72.1%)
64 (48-678 ms, 10 ms increments)	194.0 ± 4.7	2-exp, 3-par (fixed c/a = 52/48 = 1.08333) [a]	
		239.5 ± 10.3 (52.0%)	48.2 ± 9.0 (48.0%)
64 (48-678 ms, 10 ms increments)	1-exp, 3-par [b]	2-exp, 5-par [c]	
	151.7 ± 5.4	203.1 ± 18.0 (46.3%)	31.3 ± 8.6 (53.7%)

[a] Using a fixed ratio of 52% long-T_2 component and 48% short-T_2 component, after Ke et al. 2002 (7).
[b] Using a 1-exponent, 3-parameter fit ($y = y_0 + ae^{-bx}$), signal-to-noise $a/y_0 = 19$.
[c] Using a 2-exponent, 5-parameter fit ($y = y_0 + ae^{-bx} + ce^{-dx}$), signal-to-noise $(a + c)/y_0 = 78.5$.

The results in Table 6 above indicate that as few as 20 TE values are sufficient for reliable bi-exponential fitting, as long as the TE values span the entire decay curve and the other criteria mentioned above are also satisfied. It also shows the importance of having TE values short enough (in this 1.5T example, less than 60 ms; see row 6 of table) to capture the decay of the faster relaxing component. The next-to-last row of Table 6 shows results of bi-exponential fits when the proportions of the two components are fixed at 52/48, as done for Cr/PCr (7). The last row of Table 6 shows the results from a 1-exponential, 3-parameter fit equation and a 2-exponential, 5-parameter fit equation. Including the finite baseline parameter y_0 rather than forcing decay to zero can improve the fit to the data in cases of inadequate SNR. The results of

the last row of Table 6 indicate that although this is the case for the Ke et al. (7) data, a bi-exponential decay is still obtained.

It has been pointed out (12) that non-monoexponential decay of diffusion attenuation curves can arise from two populations within the same cellular compartment with different diffusion coefficients, from two cellular compartments with different environments conferring different diffusion coefficients to species within them, or from restricted diffusion within a compartment. The first two situations are analogous (a) to PCr and Cr within the same tissue type and/or cellular compartment having different T_2 values and (b) to tCr having different T_2 values in different tissue types (e.g., GM and WM) and/or cellular compartments (e.g., cytoplasm and mitochondria) but with the two molecular species having indistinguishable T_2 values within a specific tissue type or cellular compartment. Even in normal variable TE experiments with no diffusion gradients applied, either with a single or with multiple variable gradient strengths or durations, using increments of echo spacing in a single-echo experiment to vary TE, rather than different numbers of echoes with a constant echo spacing, can in theory reduce the ability to detect bi-exponential decay in cases where there are inherent microscopic gradients of the magnetic field within the cell through which the species are diffusing. This is because in the single-echo experiment, with variable echo spacing used to generate different TE values, successively longer echo spacing will include increasingly more signal decay from diffusion through the microscopic gradients, for unrestricted diffusion. It is thus apparent that a series of carefully planned and executed experiments are necessary to distinguish which of the several different circumstances are occurring before unambiguous interpretations of the data are possible, especially in a complex biological system in vivo. This is further complicated in the case of the methyl peaks of tCr because other species, especially GABA and macromolecules, have resonances in the 3.0 ppm region which overlap those of PCr and Cr in the ^{1}H spectrum.

Another series of data analyses needs to be done to test the effect of macromolecular (MM) overlap on the 3.0 ppm tCr methyl resonance, by subtracting the MM contribution, as mentioned by Ke et al. (7), following Behar and colleagues (19, 20) The GABA contribution to the 3.0 ppm resonance region has been calculated to be <5%, negligibly small (7).

Task 4. Recruitment and scheduling of Gulf War veterans (**months 5-24**)
4a. Recruit and schedule Gulf War veterans from the national sample of 97 Gulf War veterans tested in 2009-2010 (**months 5-23**).

- Work on this task was begun, but then halted due to continuing technical issues (see above Tasks 2 and 3).

4b. Make financial payments and expense reimbursements (**months 5-24**).

- Work on this task has not yet begun.

Task 5. Collection of ^{1}H and ^{31}P MRS data from Gulf War veterans (**months 6-23**)
5a. Year 1 target is collection of data from 31 veterans (**months 6-12**).

- Work on this task has not yet begun.

5b. Year 2 target is collection of data from an additional 66 veterans (**months 13-23**).

- Work on this task has not yet begun.

Task 6. Analysis and reporting of ^{1}H and ^{31}P MRS data from Gulf War veterans (**months 6-24**)
6a. Transfer ^{1}H and ^{31}P MRS data to an off-line computer for analysis and archiving (**months 6-24**).

- Work on this task has not yet begun.

6b. Interpret the data, relate it to prior data, and write reports and papers (**months 13-24**).

- Work on this task has not yet begun.

Problem Areas

A. ^{31}P Performance of Dual-tuned ^{31}P-^{1}H Head Coil

There was a temporary problem with ^{31}P coil performance when the dual-tuned ^{31}P-^{1}H head coil was first delivered that persisted after it was sent back to the manufacturer for repair and returned to UT Southwestern. Cable connections in the 3T Philips MR system were found to be faulty; when this was fixed, the circumstances improved but intermittent SNR deficiencies still remained. The coil was again sent back to the manufacturer and a faulty connector in the transmit/receive (T/R) switch was discovered and fixed, eliminating the intermittent issues.

B. Ability to Detect Individual Cr and PCr T_2 Components with ^{1}H MRS

A potential problem exists with the planned measurement of bi-exponential relaxation of the tCr peak with ^{1}H MRS to resolve Cr and PCr. This approach was proposed based upon the reported nearly three-fold (117 ms for PCr and 309 ms for Cr, measured at 1.5T with 64 TE values) difference in T_2 values for the overlapping PCr and Cr methyl peaks constituting the tCr methyl resonance (7). However, a more recent experiment by the same laboratory (9) performed at 4T with 48 TE values failed to detect the individual Cr and PCr transverse relaxation times, instead measuring only a single averaged component as is typically reported in the literature. This failure was blamed upon the fewer TE values used compared to the original report that successfully obtained individual Cr and PCr T_2 values. As demonstrated above, it is likely that SNR rather than fewer TE values was limiting in the experiments reported in the later paper (9), which used much smaller voxels.

The inability of Ongur et al. (9) to reproduce the findings of Ke et al. (7) spurred us to investigate the equations governing the transverse relaxation of Cr and PCr under conditions of chemical exchange mediated by creatine phosphokinase (CPK), as occurs *in vivo*, using realistic parameters from the literature to determine the theoretical feasibility of detecting and measuring the two individual Cr and PCr T_2 relaxation components.

There are two questions of interest in the following calculations pertinent to Cr and PCr. The first and most important, from the perspective of our proposed experiments, is whether or not the PCr ↔ Cr exchange rates are fast or slow on the spectroscopic (compared to the separation of the Cr and PCr methyl peaks) and transverse relaxographic (compared to the sum of the transverse relaxation rates of the two exchanging species) time scales. This will dictate whether both individual transverse relaxation components can be measured (slow regime) or whether a single average transverse relation rate is measured (fast regime). The second is whether the exchange contribution to the transverse relaxation is insignificant or not; if it is not insignificant, then it might be desirable to use a very short echo spacing (τ_{CPMG}) to effectively remove its contribution, or to vary τ_{CPMG} or use a very short and a very long value of τ_{CPMG} to quantify the contribution of the exchange rate to the overall transverse relaxation.

The measured transverse relaxation rate $R_{2,CPMG}$ measured as a function of the Carr-Purcell-Meiboom-Gill (CPMG) echo spacing τ_{CPMG} is given by the Luz-Meiboom (LM) equation (21):

$$R_{2,CPMG} = A + B[1 - (2C/\tau_{CPMG}) \tanh(\tau_{CPMG}/2C)]$$

where $A = R_{2M}$ is the observed or measured (average) transverse relaxation rate absent exchange (or in the presence of exchange but when τ_{CPMG} is so short as to approach zero, thus effectively removing the exchange contribution from observation), $B = P_A P_B(\Delta\omega)^2 \tau_{ex}$ is the exchange contribution to the measured transverse relaxation rate, and $C = \tau_{ex}$ is the exchange lifetime. In the expression for B, P_A and P_B are the relative populations of the two exchanging species (here Cr and PCr) and $\Delta\omega$ is the separation (in Hz or s^{-1}) between the Cr and PCr methyl peaks in the ^1H spectrum.

CPK-mediated exchange between Cr and PCr on the spectroscopic time scale. The relative magnitudes of the spectroscopic shutter-speed $\Delta\omega \equiv |\omega_A - \omega_B|$, the frequency separation in Hz of two peaks in an MR spectrum, and of the exchange rate constant τ^{-1}, where $\tau^{-1} \equiv \tau_{PCr}^{-1} + \tau_{Cr}^{-1}$ and τ_{PCr} and τ_{Cr} are the lifetimes of the methyl spins in the two sites, dictate whether two peaks with separately measurable relaxation times are observable or a single merged peak is observed (22). If $\Delta\omega >> \tau^{-1}$ (the slow-exchange-limit or SXL condition, or the no-exchange-limit or NXL condition), two peaks are seen. In the intermediate exchange regime ($\Delta\omega \approx \tau^{-1}$), two partially overlapping peaks are detected. If $\Delta\omega << \tau^{-1}$ (the fast-exchange-limit or FXL condition), one spectroscopic peak (resonance frequency) is seen.

The separation $\Delta\omega$ between the PCr and Cr ^1H methyl peaks is 0.0020 ppm (23), which in frequency units is 0.128 Hz at 1.5T, 0.255 Hz at 3T, 0.340 Hz at 4T, and 0.596 Hz at 7T. By comparison, the forward rate constant k_f(PCr→Cr+ATP) for the CPK-mediated reaction in human brain is 0.3 s^{-1} (24-26) and the reverse rate constant k_r(Cr+ATP→ PCr) is 0.42 ± 0.05 s^{-1} (25). The exchange rate term $1/C = 1/\tau_{ex}$ in the LM equation equals k_f(PCr→Cr+ATP) + k_r(Cr+ATP→ PCr) which sums to 0.72 $^{s-1}$. Thus the exchange regime is intermediate-fast for low field (1.5T) and intermediate for intermediate field (3T, 4T) and for high field (7T). These calculations indicate that, on the spectroscopic time scale, it would be difficult to resolve the individual PCr and Cr methyl peaks to measure their separate transverse relaxation components unless an ultra-high field magnet (>10T) were used. This is well-know and obvious from experimental spectra.

CPK-mediated exchange between Cr and PCr on the transverse relaxation time scale. Even if two spectral peaks are superimposed and can't be resolved, as is the case for the PCr and Cr methyl peaks at practically available field strengths, separate relaxation times T_1 and/or T_2 can still be observed as a bi-exponential relaxation curve under appropriate circumstances. This has been explained in terms of the relaxographic shutter speed, τ_X^{-1}, defined for the case of PCr and Cr as $|R_{XPCr} - R_{XCr}|$, where $R_X \equiv 1/T_X$, and X = 1 for longitudinal relaxation and 2 for transverse relaxation (12, 27-29). The recovery exponentiality depends on the relative magnitudes of τ_X^{-1} and the exchange rate constant, τ^{-1}, where $\tau^{-1} \equiv \tau_{PCr}^{-1} + \tau_{Cr}^{-1}$ and τ_{PCr} and τ_{Cr} are the lifetimes of the methyl spins in the two sites. If $\tau_X^{-1} << \tau^{-1}$ (the FXL condition), the relaxation will be averaged and the recovery will be mono-exponential. If $\tau_X^{-1} >> \tau^{-1}$ (the SXL condition or the NXL condition), the recovery will be non-mono-exponential and individual relaxation components can be measured. Transverse shutter-speed (τ_2^{-1}) values are typically larger than longitudinal shutter-speed (τ_1^{-1}) values and thus, in general, it is easier to depart the FXL condition into non-mono-exponentiality for T_2 than for T_1.

Multiple single-site ^{31}P saturation magnetization transfer experiments of human occipital lobe performed at 7T yielded CPK exchange rate constants of $k_f = 0.30 \pm 0.04\ s^{-1} = \tau_{PCr}^{-1}$ for PCr + ADP + H^+ → Cr + ATP and $k_r = 0.42 \pm 0.05\ s^{-1} = \tau_{Cr}^{-1}$ for Cr + ATP → PCr + ADP + H^+ (25). Thus $\tau^{-1} = \tau_{PCr}^{-1} + \tau_{Cr}^{-1} = 0.30\ s^{-1} + 0.42\ s^{-1} = 0.72\ s^{-1}$. From the literature report of bi-exponential

tCr T_2 behavior at 1.5T in human brain (7), Cr T_2 = 309 ± 21 ms and PCr T_2 = 117 ± 21 ms; thus the relaxographic shutter speed, $\tau_2^{-1} = \left| R_{2PCr} - R_{2Cr} \right| = \left| 8.55\ s^{-1} - 3.24\ s^{-1} \right| = 5.3\ s^{-1}$. Since τ_2^{-1} (= 5.3 s^{-1}) >> τ^{-1} (= 0.72 s^{-1}), this is consistent with the theory predicting SXL and bi-exponential behavior.

Contribution of exchange to the transverse relaxation. At 1.5T in human brain, T_2 has been reported to be 309 ± 21 ms for Cr, 117 ± 21 ms for PCr, and 222 ± 14 ms for the averaged tCr T_2 (7). So the average transverse relaxation rate absent exchange $A = R_{2M} = tCr\ R_2 = (tCr\ T_2)^{-1} = (0.222\ s)^{-1} = 4.5045\ s^{-1}$. For human brain, $P_{PCr} = 0.48$ and $P_{Cr} = 0.52$, so $P_A \approx P_B$ (7 and references therein). Since $\tau_{ex} = C = 1/\ k_f(PCr \rightarrow ATP) = 1/0.3\ s^{-1} = 3.3$ s, the exchange contribution to the transverse relaxation rate $B = P_A P_B (\Delta\omega)^2\ \tau_{ex} = (0.48)(0.52)(0.128\ s^{-1})^2(3.3\ s) = 0.0135\ s^{-1}$. Because A (= 4.5045 s^{-1}) >> B (= 0.0135 s^{-1}), the exchange contribution to transverse relaxation is negligible. The same holds true at 3T (A (= 7.97 s^{-1}) >> B (= 0.054 s^{-1}), 4T (A (= 6.85 s^{-1} for ACC and 8.06 s^{-1} for POC) >> B (= 0.0952 s^{-1}), and 7T (A (= 11.11 s^{-1}) >> B (= 0.2926 s^{-1}). From UTSW data at 3T for basal ganglia, tCr T_2 = 125.5 ± 7 ms so $A = R_{2M} = (0.1255\ s)^{-1} = 7.97\ s^{-1}$. For 4T, tCr T_2 = 146 ± 22 ms in ACC and in 124 ± 29 ms in POC, so $A = R_{2M} = (0.146\ s)^{-1} = 6.85\ s^{-1}$ for ACC and for $(0.124\ s)^{-1} = 8.06$ s-1 for POC (7). From literature data for basal ganglia at 7T (30), tCr T_2 = 90 ± 11 ms so $A = R_{2M} = (0.090\ s)^{-1} = 11.11\ s^{-1}$.

The section two paragraphs prior indicates that for human brain in vivo, the CPK reaction exchanging PCr and Cr is likely in the SXL and thus bi-exponential behavior should be observable if other conditions are optimal (10-12), particularly if there is at least a three-fold difference in relaxation times and if 20 > SNR > 50 (or more). There are two alternatives to PCr and Cr bi-exponential T_2 determinations: (1) Chemical exchange saturation transfer (CEST) with ^{1}H imaging may be an alternate way to estimate Cr and PCr (31). However, this is a new method only recently introduced and feasibility tested only for Cr. It would require time to implement and test this technique for both Cr and PCr, which would delay the project, even if proven feasible for both. Thus it practical to defer this effort to investigate CEST until later, making it the topic of a future, separate grant application and project. (2) ^{31}P magnetization transfer studies of brain CPK and APTase kinetics would complement and augment the ^{31}P MRS studies of brain that were originally proposed for this project. This alternative is attractive because it would make use of the ^{31}P-^{1}H dual-tuned head coil, which is not commonly available at most MR sites. However, the PI is moving to a new institution with a 3T Siemens MR system lacking multinuclear capability, so the ^{31}P experiments will not be possible there. Instead, multi-TE experiments will be done as originally proposed, in an effort to measure bi-exponential decay of the tCr methyl signal and determine PCr and Cr T_2 values.

C. Available Time on and Access to UT Southwestern AIRC 3T Philips MR System

When planning for this project began, time was much more available on the UT Southwestern AIRC 3T Philips MR system than now. Reserving time has been more difficult and challenging than anticipated when the accelerated two-year grant period was chosen. To help this situation, the ^{1}H protocol was tested and set up on both AIRC 3T Philips systems. Because of the problems with the dual-tuned ^{31}P-^{1}H coil and with getting time on the AIRC 3T systems, the project is now 6 months behind schedule. Adding several months of expected delays from the PI's impending move to another institution, the performance period needs to be extended a year, making its duration three rather than two years. Annual personnel effort and percentages of salary support will be adjusted in the re-budget for the new institution to extend over the longer grant period.

Recommended Changes or Future Work To Better Address the Research Topic

Given the delays incurred by the difficulties described above, it is now certain that more time than the two-year grant period requested will be necessary to complete the study. Therefore, a one-year no-cost extension with re-budget will be requested as part of the grant transfer from UT Southwestern to Georgia State University. The P.I. has spoken with the CDMRP Research Program Manager/Science Officer/Grants Officer Representative to explain that the re-budget is necessary so that personnel and other costs can be re-distributed over the three-year period over which work is now proposed to be done.

Partly due to the results obtained to date and partly due to the P.I.s impending move to another institution, several alterations to the original scientific plans will be necessary, and will be included in the upcoming submission of a revised Statement of Work.

First, since Georgia State University (GSU) has a 3T Siemens rather than a 3T Philips MR instrument, ^{31}P MRS will not be possible at GSU, since the dual-tuned ^{31}P-^{1}H coil works only on 3T Philips systems. The P.I. investigated the possibility of leaving the data collection at UT Southwestern, so that ^{31}P MRS could be done, but that is not feasible. The lower than expected ^{31}P MRS sensitivity of the dual-tuned coil compromises the proposed ^{31}P MRS studies in several ways: (a) using shorter TR, 2-3 s rather than the 20 s needed for complete T_1 relaxation, conflates relaxation effects with concentration in the signal intensity; (b) more signal averages are needed, taking significantly longer; (c) larger voxels (at least 100 mL), are required, making volume averaging of gray matter and white matter and of different brain regions a problem, and precluding collection of data from the important basal ganglia region, as originally proposed. Omitting the compromised ^{31}P MRS allows opportunity and time to replace it with additional ^{1}H MRS experiments that have a better chance of yielding useful results, under these circumstances.

Second, for ^{1}H MRS, spectra from three additional brain regions likely to be damaged in GWI can be done in the time saved from the omission of ^{31}P MRS: white matter in centrum semiovale, anterior cingulate cortex (ACC), and cerebellum. The rationales for adding these three additional important voxels are as follows. First, abnormalities in white matter have been reported in veterans with Gulf War Illness (3-6) and in those exposed to sarin gas in the 1995 Tokyo subway attack (32). Second, from an organophosphate toxicity study, only striatum and hippocampus have higher acetylcholinesterase (AChE) activity than cerebellum in rat brain (2). Third, ACC is functionally related to several symptoms of GWI, and lactate (Lac) and glutamate/glutamine (Glx) changes with exercise differ in two groups of ill Gulf War veterans also symptomatic for chronic fatigue syndrome that respond differently to exercise stress (1). Yet these three regions have not yet been interrogated with MR spectroscopy in Gulf War Illness. In addition, fully relaxed ^{1}H spectra (TR = 8s rather than the TR = 3s originally proposed) can be collected, removing the potential confound of longitudinal relaxation conflating with concentration in ^{1}H signal intensities, present in all previous ^{1}H MRS studies of Gulf War Illness.

Our preliminary theoretical and experimental results indicate that it will be difficult to measure bi-exponential decay of the tCr methyl signal in multi-TE experiments to measure T_2, but have defined the 3T parameter ranges for which the chances of doing so are optimized. Therefore we intend to collect this data as originally planned and proposed. In the worst case, this will allow more accurate determination of tCr T_2 values than in our previous studies, and will shed more light on the feasibility of measuring bi-exponential tCr (Cr + PCr) T_2.

Documents, including a revised Statement of Work, to transfer the grant with the PI from UT Southwestern Medical Center in Dallas to Georgia State University in Atlanta are currently being prepared for submission to the Army Contracting Officer/ Grants Specialist Representative and the CDMRP Research Program Manager/Science Officer/Grants Officer Representative for their approval. The PI has been communicating with them both, as well as with grants offices of his present and future institutions, to ensure that proper procedures are followed.

Key Research Accomplishments

- Trouble-shooting tests were done to get the ^{31}P coil of the dual-tuned ^{31}P-^{1}H head coil to perform reliably, and experiments were conducted to develop a ^{31}P MRS protocol and optimize parameters.
- Sequences were developed and parameters were optimized for the ^{1}H MRS protocol, including voxel locations and sizes.
- Theoretical calculations performed using parameter estimates from the literature indicated that the CPK-catalyzed chemical exchange of phosphate between PCr and Cr at 3T is in the fast exchange limit on the spectroscopic time scale and in the slow exchange limit on the transverse relaxation time scale, and that the exchange process contributes negligibly to the ^{1}H 3.0 ppm tCr methyl peak transverse relaxation.
- Experiments to define the best 3T parameters for measuring bi-exponential T_2 relaxation of the methyl peak of tCr at 3.0 ppm indicated that (1) TEs shorter than about 40 ms are needed to adequately quantify the faster relaxing component; (2) as few as 20 TE values are sufficient for reliable bi-exponential fitting, as long as the TE values span the entire decay curve and adequate SNR (20-50) is obtained; (3) including a finite baseline parameter y_0 rather than forcing decay to zero may improve the fit to the data in cases of marginal SNR. The first result suggests that STEAM, which can allow substantially shorter TEs than PRESS, may be advantageous despite its lower sensitivity per unit time. To ensure that GM-WM T2 differences are not confounding, data from a voxel in WM centrum semiovale will be used for the T_2 relaxation time determinations.

Reportable Outcomes

- No manuscripts, abstracts, or presentations have yet resulted from this research;
- No licenses have been applied for or issued;
- No degrees have been obtained that are supported by this award;
- No cell lines or tissue or serum repositories have been developed;
- No informatics such as databases or animal models have been developed;
- No funding has been applied for based on work supported by this award;
- The P.I. has accepted a new position at Georgia State University in Atlanta to begin November 1, 2013 based in part on this award and the experience it is providing, as well as the P.I.'s former experience applying MR techniques to Gulf War Illness and other neurological disorders. In addition, the P.I. applied for a position at the University of Florida and the VA Medical Center in Gainesville, Florida and was actively recruited

there, based in part on experience doing Gulf War Illness research supported by this award and previous funding from the VA and DoD.

Conclusion

After the initial year of the project, a number of important research objectives have been accomplished (see "Key Research Accomplishments" section above). Despite these achievements, the project is about 6 months behind schedule and no Gulf War veterans have yet been studied because of delays related to technical problems with the dual-tuned ^{31}P-^{1}H head coil. These problems have now been solved; however, since the P.I. is moving to Georgia State University in Atlanta on November 1 and will be using a 3T Siemens system there that is not compatible with this coil, the coil will not be used to collect ^{31}P data. Transfer of the grant there will include a request for a one-year no-cost extension.

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Appendices

Appendix 1. Hart 10/07/13 Data/Safety Monitor Letter

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October 7, 2013

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Dear Richard,

I have reviewed the subject enrollment report and demographic information for the 8 normal volunteer controls studied in 24 MR session during Year 1 of your DoD grant W81XWH-12-1-0321, application GW110034, "Abnormalities in Human Brain Creatine Metabolism in Gulf War Illness Probed with MRS" to test equipment, optimize the protocol and parameters, and obtain preliminary normative transverse relaxation data.

There were no adverse events, and no subjects reported any unexpected or severe discomforts during the scanning sessions. Two subjects did move around somewhat during their sessions, some of which were fairly long (about 1.5 hours), and one asked to be removed about ten minutes before the scanning was completed to go to the rest room. This is not out of the ordinary. There were no protocol deviations or violations, no participant withdrawals or dropouts, and no changes in risk/benefit ratio. No unanticipated abnormalities were observed in the images, and so the services of a clinical neuroradiologist were not needed, since no unusual features that required medical follow-up were noted.

The study is about six months behind schedule and no GW veterans have been studied yet, due to unanticipated and recurrent problems getting the

dual-tuned 31P-1H 3T head coil to function properly in the 3T Philips Achieva in the Advanced Imaging Research Center at UT Southwestern. Those issues have been solved and both ^{1}H and ^{31}P MRS parameters are now optimized and the protocols fully tested and finalized. Thus, I note no issues and everything seems to be going well at present.

Sincerely,

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